



TITLE:

# Manufacture of Gluconic Acid by Fermentation under Aeration. : On the Industrial Studies of Oxidising Fermetation

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(8) Diastase was precipitated by tannic acid from crude extract [I] of dried malt, and this precipitate was washed by alcohol [II] according to the patent. Further purification [III] was carried out as follows: the filtrate of aqueous solution of [II] was dried under reduced pressure (1/100~2/100 mm Hg) at  $-70^{\circ}\text{C}$ .

When these preparations are compared with the precipitate [IV] obtained by alcohol as is generally performed, it will be seen in the Table that the preparation [III] showed the highest diastatic power and preparation [II] is superior to preparation [IV] both in the yields and diastatic power.

Preparation	Diastase unit of pharmacopoeia	Yield (%)	Saccharifying power		Starch liquefying power	Proteoclastic power
			$\alpha$ -Amylase	$\beta$ -Amylase		
I	0.3	100	0.12	1.00	1.0	1.0
II	14.3	59	6.24	48.13	42.7	29.3
III	22.0	50	8.64	85.36	64.0	58.2
IV	9.0	54	5.25	41.26	45.7	19.4

(9) The enzyme compositions of these preparations were compared with each other in the following ways:  $\alpha$ - and  $\beta$ -amylase were determined by Kneen and Sandstedt's method,<sup>2)</sup> starch liquefying power was observed by viscosimeter with potato starch solution and proteoclastic power upon gelatin was determined by formol titration method. It will be pointed out that, in the above Table, the highest powers of these enzymes were found in preparation [III], and preparation [II] revealed higher enzymatic powers with little liquefying power when it was compared with preparation [IV].

No remarkable difference in the ratio of saccharifying power to liquefying power was observed among all these preparations, however proteoclastic power was found to decrease by the treatments of precipitation especially with alcohol.

- 1) Katagiri, Shibutani and Mugibayashi: This Bulletin 18, 45 (1949), 19, 61 (1949).
- 2) Kneen and Sandstedt: Cereal Chem. 16, 712 (1939), 18, 273 (1941).

### 35. Manufacture of Gluconic Acid by Fermentation under Aeration.

On the Industrial Studies of Oxidising Fermentation

*Hideo Katagiri and Koji Itagaki.*

In order to prepare gluconic acid from glucose, several experiments were carried out as to determine a suitable source of nitrogen substances, and wheat bran Koji extract was found to be practically useful among various kinds of materials, such as wheat bran, soy bean cake, yeast and beer spent Koji.

With the medium composed of glucose and wheat bran Koji extract, a strain of *Gluconobacter* isolated from fruit was chosen as the best among twelve strains of *Acetobacter*. 1~1.5 L of the medium was put in a cylinder (10 cm diameter, 60 cm depth), mounted with a glass filter (No. 4) near the bottom and fermentation was carried out under aeration of 1~1.2 L air per minute. Alcoholic solution of Cetyl alcohol was used as anti-effervescent, and the changes of pH and titration acidity were observed every 5~6 hours.

Various kinds of experiments were carried out, in order to ascertain suitable conditions for fermentation, and a successful result was obtained when the medium contained 5 % wheat bran Koji extract and 10 % glucose. Fermentation was accelerated when 0.1 % urea and 2.5 % wheat bran Koji were used as the source of nitrogen. The optimum pH of the medium was found to be 3.8, although pH value did not reveal any remarkable effect upon the fermentation up to 5.5. Suitable amount of bacterial inoculation was found to be 10 % of the medium, and commercial glucose was available for industrial fermentation, while better yield was obtained with purified glucose.

The maximum yield of gluconic acid was observed to be 101.7 % of the glucose employed, under the above experimental conditions.

When the yield of bacterial cells and the amount of nitrogen assimilated by the bacteria were observed every 1~5 hours during the fermentation, it was found that velocities of propagation of the bacteria and assimilation of nitrogen were accelerated by aeration.

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### **36. On the Antibacterial Properties of Retting Bacteria.**

*Hideo Katagiri and Shinzo Kohno.*

The general industrial process of fermentation-retting of ramie fiber materials has been carried out with open vessel, and yet fermentation has been smoothly going without any troublesome contamination.

This fact suggests that the retting bacteria for ramie fiber materials, *Bacillus subtilis* var. *ramie* would produce some antibiotics as were pointed out with other spore-bearing aerobes, such as *B. subtilis*, *B. mesentericus*, *B. mycoides*, *B. simplex*, *brevis B.* etc.

The antibacterial action of the retting bacteria on *Staphylococcus aureus* (Terashima strain) and *Escherichia coli* was detected by (1) method of plate culture on bouillon agar, and (2) cup method with bouillon added by fiber materials of wild ramie plant.